Loss of proximal tubular Krüppel-like factor 15 (KLF15) exacerbates kidney injury through loss of fatty acid oxidation

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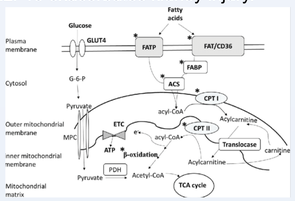
ABSTRACT

Loss of fatty acid β-oxidation (FAO) in the proximal tubule (PT) is a critical mediator of acute kidney injury (AKI) and eventual fibrosis. However, transcriptional mediators of FAO in the setting of PT injury remain understudied. Krüppel-like factor 15 (KLF15), a highly enriched zinc-finger transcription factor in the PT, was significantly reduced in PT cells after aristolochic acid I (AAI) treatment. PT-specific knockdown of *Klf15 (Klf15*^{PTKO}) exacerbated kidney function as compared to control (*Klf15*$^{fl/fl}$) mice during AAI treatment. RNA-sequencing of kidney cortex demonstrated a decrease in transcripts encompassing FAO and PPARα, a transcription factor that regulates FAO genes, in *Klf15*^{PTKO} versus *Klf15*$^{fl/fl}$ mice after AAI treatment. Thus, PT-specific loss of *Klf15* exacerbates AKI and fibrosis, likely due to loss of interaction with PPARα leading to loss of FAO gene transcription.

BACKGROUND

Uninjured PT cells mainly use FAO for their ATP production, and inhibition of this process using carnitine palmitoyl transferase (CPT1) inhibitor, etomoxir, can induce dedifferentiation and apoptosis of tubular cells *in vitro* and worsen folic acid-induced kidney injury *in vivo*.[1] Conversely, upregulation of FAO by overexpressing peroxisome proliferator activated receptor alpha (PPARα) agonist, attenuates kidney injury.[2] In previous studies, KLF15 was shown to interact with PPARα to transcribe FAO genes in cardiomyocytes which also utilize FAO for cellular ATP production.[3] KLF15 also plays a role in kidney interstitial fibrosis and glomerulosclerosis. In nephrectomized rats, KLF15 expression was downregulated in podocytes and stromal specific loss of KLF15 exacerbated myofibroblast proliferation and interstitial fibrosis.[4] Given the protective effects of podocyte and stromal KLF15 in kidney injury and the potential role of KLF15 in regulation of FAO, we hypothesize that loss of PT-specific KLF15 exacerbates kidney injury.

Fig 1. PPARα regulates fatty acid utilization and β-oxidation.
Stars indicate transporters and enzymes involved in FAO which are regulated by PPAR-α.



RESULTS

I: KLF15 overexpression in PT with doxycycline & downregulation with cisplatin-induced injury

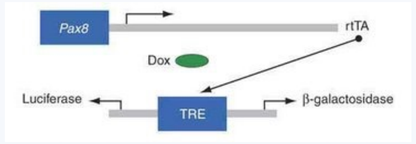


Fig 2. Doxycycline Mechanism.
Pax8—rtTA mice generated & Tet-On tool used.

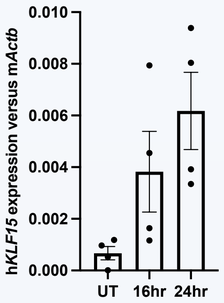


Fig 3. Overexpression of hKLF15 with doxycycline

Primary PT cells were derived and 1μg/mL doxycycline was added 16 and 24 hours prior to measurement. qPCR result shows ten-fold increase of hKLF15 expression after 24 hours of treatment compared to untreated cells.

Challenge: Ideally want greater fold of increase with less variability. Primary PT cell derivations tend to be affected by different backgrounds of mice. Next step is to confirm growth of primary PT cells and induce injury with toxin such as cisplatin to confirm downregulation of KLF15 expression.

Confirmation of Primary PT cells with Immunostaining

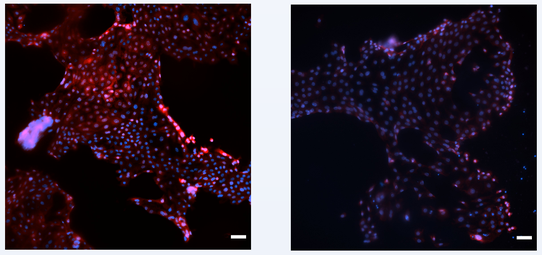


Fig 4 and 5. Validation of KLF15 expression in PT cells—1:100 and 1:200 dilutions of KLF15 respectively.
Immunofluorescent staining for KLF15 (pink), with counterstaining for Texas red (red) and DAPI (blue).

II: Metabolic Live Tissue Assay with Compresstome

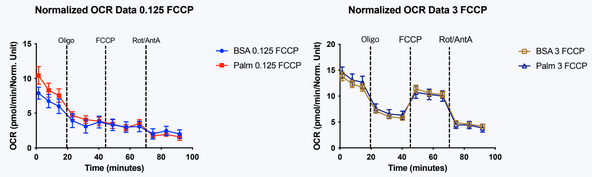


Fig 6&7. FCCP optimization at 0.125μM and 3μM respectively.
Seahorse assay measures mitochondrial function via oxygen concentration (OCR). Four compounds are added at different times, and corresponding changes in OCR are observed to evaluate mitochondrial respiration of a cell. FCCP, an oxidative phosphorylation uncoupler, transports protons across inner membrane to max the respiration rate. Optimization of FCCP amount was necessary to measure maximal respiration.

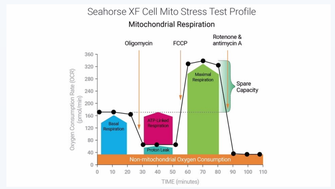


Fig 8. Seahorse Mito Stress Test Profile

Challenge:
1. Cells do not stick to plate
2. Aspirator tip too big—sucks up cells in the middle
3. Time-consuming: 1 week to grow primary PT cells

Live Tissue Seahorse Assay with Compresstome VF-310-0Z

Goal: to place thinly-sliced kidney pieces directly onto seahorse plate
Challenge: can't get flat, thin surface below 125 μm with **Vibratome**

Compresstome

1) Agarose Embedding
- Stabilize tissue
- Protect from damage

2) Auto Zero-Z blade
- Prevent z-axis deviation
- Flat surface

Findings:
- Variable depending on temperature, hardness, orientation of kidney
- Cold, hard, vertical kidney works the best
- Pre-chill buffer solution
- Agarose falls out easily; leave in chilling block for 4-5min
- Best at speed 4 & oscillation 10

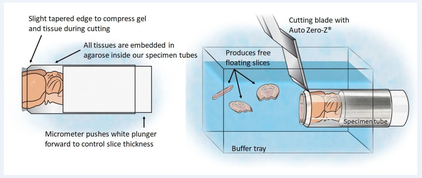


Fig 9. Compresstome Animation

CONCLUSIONS

In this study, we demonstrate the physiological role of PT-specific KLF15 in the setting of DNA damage-induced injury. KLF15 is downregulated during injury, and we show that this is a maladaptive response, since *Klf15*^{PTKO} mice had exacerbated injury in the active phase of AAI toxicity, which also led to worsened fibrosis. We previously demonstrated the role of KLF15 in podocyte injury and in stromal cells during fibrotic injury, but this is the first report showing the role of KLF15 in the PT using a PT-specific injury model. Furthermore, we use doxycycline to overexpress hKLF15 and the next step is to induce downregulation with PT-specific toxin such as cisplatin. We also show by live cell metabolic assays in primary PT cells that the importance of KLF15 is at least partly due to its transcriptional regulation of genes encoding the key FAO enzymes CPT1 and ACAA2, which are critical to maintaining PT cellular metabolism, and this effect is likely through co-operation with PPARa. In conclusion, downregulation of PT-specific *Klf15* during kidney injury is a maladaptive response, likely through loss of transcriptional activation of essential FAO genes in conjunction with PPARa. This effect is consistent across nephrotoxic, HIV-associated, and UUO nephropathies, and in human HTN and DKD patients.

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